16.5%; OR=1.52, 95%C.I., 1.10–2.11; $P=0.010$), while the CGC haplotype was associated with a decreased risk of metabolic syndrome, also only in men (metabolic syndrome 63.6%, control 70.3%; OR=0.73, 95%C.I., 0.56–0.96; $P=0.026$). After permutation tests ($n=1000$), the association between the GAC haplotype and metabolic syndrome remained significant ($P=0.046$) while that between CGC and metabolic syndrome did not ($P=0.094$).

The −358A allele is a representative SNP of the GAC haplotype of SNP-1, -2, and -3 and contributes to an increased risk of metabolic syndrome. Assuming a dominant model, the −358A polymorphism was associated with an increased risk of developing metabolic syndrome in Japanese individuals (OR=1.3, 95%C.I., 1.0–1.8; $P=0.047$). However, as shown in Table 4, the −420G allele had a higher odds ratio in metabolic syndrome subjects than controls after adjusting for sex and age (OR=1.5, 95%C.I., 1.1–2.0; $P=0.004$). Moreover, the +299G>A SNP in intron 2 was also dominantly associated with metabolic syndrome in this Japanese cohort. As the +299G>A SNP was not in linkage disequilibrium with either SNP-420C>G or SNP-358G>A, the +299A allele may be another putative marker SNP for metabolic syndrome that is unrelated to the −420C>G SNP. However, there was no significant association with the +299A allele in both men and women subgroups. Moreover, the −420C>G SNP and −358G>A SNP were significantly associated with metabolic syndrome only in men.

Association of the −420C>G SNP with clinical parameters in urban Japanese men and women

Table 5 shows the association of these SNPs with various clinical parameters using an analysis of covariance, after adjusting for age. Diastolic blood pressures in men with the −420CG+GG genotype were significantly higher than those in men with the −420CC genotype. Men with the −420CG+GG genotype also had higher serum triglyceride levels and lower serum HDL cholesterol levels than those with the −420CC genotype. Insulin resistance by the homeostasis model of assessment (HOMA-IR) value was significantly higher in those with the −420CC+CG genotype than in those with the −420CC genotype (1.66±0.02 vs. 1.50±0.06; $P=0.043$). Moreover, serum adiponectin levels in men with the −420CG+GG genotype were significantly lower than levels in men with the −420CC genotype.

Table 5 Comparison of clinical parameters in urban Japanese men and women ($n=2968$) according to resistin −420C>G genotype.

	Men ($n=1354$)			Women ($n=1614$)		
	CC ($n=591$)	CG+GG ($n=763$)	P	CC ($n=694$)	CG+GG ($n=920$)	P
Age	68.3 ± 10.6	67.0 ± 10.7	0.026	64.5 ± 11.1	63.8 ± 10.9	0.165
BMI	23.0 ± 0.1	23.3 ± 0.1	0.081	22.3 ± 0.1	22.4 ± 0.1	0.340
Waist (cm)	85.3 ± 0.3	85.8 ± 0.3	0.290	83.3 ± 0.4	83.2 ± 0.3	0.877
SBP (mmHg)	131.9 ± 0.8	133.2 ± 0.7	0.215	130.6 ± 0.8	129.7 ± 0.6	0.364
DBP (mmHg)	78.3 ± 0.4	79.5 ± 0.4	0.028	76.5 ± 0.4	76.9 ± 0.3	0.357
FBG (mmol/l)	5.72 ± 0.06	5.78 ± 0.05	0.316	5.37 ± 0.03	5.41 ± 0.04	0.524
HbA_{1c} (%)	5.60 ± 0.04	5.62 ± 0.03	0.684	5.40 ± 0.02	5.67 ± 0.02	0.099
HOMA-IR	1.50 ± 0.06	1.66 ± 0.02	0.043	1.34 ± 0.04	1.35 ± 0.04	0.527
T-Cho (mmol/l)	5.08 ± 0.03	5.14 ± 0.03	0.125	5.63 ± 0.03	5.58 ± 0.03	0.153
TG (mmol/l)	1.25 ± 0.03	1.39 ± 0.03	0.002	1.08 ± 0.02	1.08 ± 0.02	0.985
HDLc (mmol/l)	1.45 ± 0.02	1.39 ± 0.01	0.002	1.68 ± 0.02	1.67 ± 0.01	0.894
LDLc (mmol/l)	3.05 ± 0.03	3.12 ± 0.03	0.091	3.46 ± 0.03	3.41 ± 0.03	0.160
Leptin (ng/ml)	9.2 ± 0.2	9.4 ± 0.2	0.827	14.1 ± 0.3	13.9 ± 0.2	0.578
Adiponectin (mg/ml)	7.8 ± 0.2	7.2 ± 0.2	0.009	10.7 ± 0.2	10.4 ± 0.2	0.310

SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TG, triglycerides; T-Cho, total cholesterol; HDLc, HDL cholesterol; LDLc, LDL cholesterol. Data except for age are shown as the adjusted means±S.E. These values were obtained after adjusting for age by the least squares method. Age is shown as the mean±S.D. The laboratory data reported in milligram per deciliter were converted to SI units as follows: total cholesterol, HDL cholesterol and LDL cholesterol: mg/dl × 0.02586 = mmol/l; triglycerides: mg/dl × 0.01129 = mmol/l; fasting blood glucose: mg/dl × 0.05556 = mmol/l. Data on FBG, HOMA-IR, TG, leptin, and adiponectin were transformed to natural logarithm values before analysis. Numbers of missing data in all samples were one HOMA-IR value, one LDLc level, one leptin level and 15 adiponectin levels.

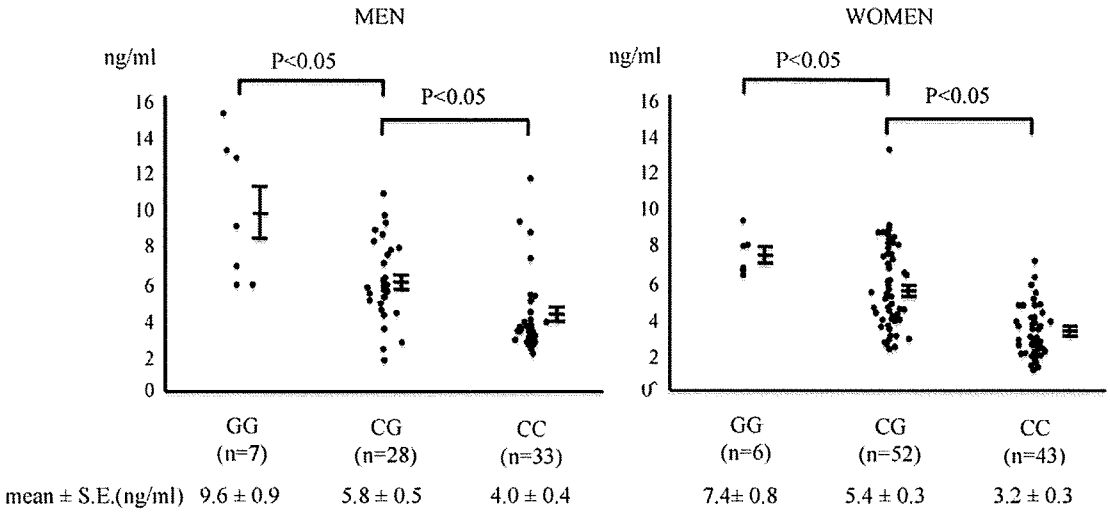

Figure 2 Plasma resistin concentration and resistin −420C > G genotype in Japanese men and women.

Association of the −420C > G SNP with plasma resistin concentration in Japanese men and women

We examined the relation between the plasma resistin concentration and the −420C > G SNP using the samples of healthy volunteers in Iwate prefecture. Fig. 2 shows plasma concentration of resistin in men and women according to the −420GG, CG and CC genotype, respectively. Plasma resistin concentration was tended to be higher in men than in women (5.3±0.3 vs. 4.6±0.3; $P=0.055$). In both men and women, plasma resistin concentration was significantly high in order of those with the −420GG, CG and CC genotype.

Discussion

This study used the 2005 Japanese definition of metabolic syndrome to diagnose and study 2968 individuals from the general population. We defined subjects having none of the components of this syndrome as controls, and thus obtained 424 metabolic syndrome subjects and 765 control subjects for the case–control study. After a thorough analysis of SNPs in the full-length resistin gene, we selected five tagging SNPs to predict haplotypes and identified one linkage disequilibrium block.

We then demonstrated that the GAC and CGC haplotypes had opposite effects on metabolic syndrome susceptibility, the former being associated with an increased risk of metabolic syndrome and the latter with a decreased risk. However, this was only true for men and there was no such association for women. We also showed that the −358A and −420G alleles, which were in linkage disequilibrium, and the +299A allele, which was not linked with the other alleles, were all associated with an increased risk of metabolic syndrome. Furthermore, the −420G allele was significantly correlated with high diastolic blood pressure, high serum triglyceride, low HDL-cholesterol and high HOMA-IR levels. Interestingly, the serum level of adiponectin (a hormone involved in insulin resistance and atherosclerosis) was also correlated with the allele, implying that these genetic variations might promote metabolic syndrome.

Previous studies on the association of resistin gene variants with obesity and type 2 diabetes have yielded conflicting results. Sentinelli et al. found no significant association of resistin gene variants in European subjects with type 2 diabetes or obesity compared to controls [15]. Osawa et al. also failed to detect an association between the −167C > T, +157C > T, and +299G > A SNPs of this gene and type 2 diabetes [16]. However, Engert et al. found an association between SNPs in the resistin gene promoter region and obesity in Canadian and Scandinavian populations [13]. Subsequently, Osawa et al. demonstrated that the CG+GG genotype of the −420C > G SNP of resistin gene is significantly associated with type 2 diabetes in Japanese subjects [21]. Additionally, they showed that this variation enhanced resistin gene promoter activity through specific binding of Sp1/3, implying that the −420C > G SNP is a causative variant [21]. We found significant associations between the prevalence of metabolic syndrome in Japanese men and resistin SNPs and haplotypes, with markedly lower *P*-values than those reported to date. Such strong associations might be due to the large sample size and the

selection of a cohort that is representative of urban Japanese populations.

Gender differences in the prevalence of metabolic syndrome have been reported [22]. Previous studies have shown that visceral fat is highly responsive to androgens, suggesting that a gender difference in the etiology of this syndrome may exist. There is also a gender difference in plasma adiponectin levels [23], and the results from this genetic study are compatible with these observations.

In mice, previous observations have suggested that resistin plays a role in insulin resistance and glucose metabolism. Banerjee et al. showed that mice with the null allele of this gene have improved glucose tolerance compared with control littermates when fed a high-fat diet [9]. This change was paralleled by decreased hepatic glucose production due to decreased gluconeogenesis. Enzymes involved in gluconeogenesis, such as glucose-6-phosphate (G6P) and phosphoenolpyruvate carboxykinase (PEPCK), had decreased activity. This reduction in activity was partly due to AMPK activation as resistin deficiency led to AMPK phosphorylation. These results suggest that resistin may enhance hepatic gluconeogenesis, presumably by antagonizing adiponectin, which inhibits enzymes involved in gluconeogenesis through AMPK activation. However, in humans, the role of resistin in insulin resistance is unclear. Fehmann and Heyn reported that plasma resistin levels are not different in type 1 and type 2 diabetes [12] and Menzaghi et al. showed the no relation to insulin resistance [24]. However, a small observational human study has indicated that serum resistin levels negatively correlate with HDL-cholesterol level, which is a component of metabolic syndrome [25].

We found that certain resistin gene variants correlated with metabolic syndrome in Japanese men. However, two limitations of this study should be noted. (1) The Japanese criteria for metabolic syndrome differ from those of the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III). Because of the cut-off value for waist circumference, a relatively small number of women were included in this study. However, the odds ratio for metabolic syndrome susceptibility in women was almost equal to the value obtained with the NCEP-ATP III criteria (data not shown). (2) The study cohort consisted predominantly of elderly Japanese men and women living in urban areas with a temperate climate. Therefore, these results need to be confirmed in other cohorts.

In summary, we found that the G allele of the −420C > G SNP of the resistin gene increased susceptibility to metabolic syndrome and correlated with the clinical traits of this syndrome. This SNP was also associated with lower serum adiponectin levels, suggesting a possible functional relevance of the *resisitin gene* in metabolic syndrome. Taken together with previous results, resistin may increase the susceptibility of metabolic syndrome by modulating lipid metabolism and adiponectin secretion from adipocytes. Further investigations are needed to confirm this hypothesis.

Conflict of interest

Authors have no competing interest in this article.

Acknowledgments

We are grateful to the following people for their support of our population survey: Dr. Yasushi Kotani, President; Dr. Katsuyuki Kawanishu, Head of the Committee for the city health check service; and other members of the Suita City Medical Association. We also thank the members of Satsuki-Junyu-kai for their cooperation and support of our survey of risk factors and prevention of cardiovascular disease. We also thank Mrs. Yae Takahashi and the members of Yahaba town.

Sources of funding: This study was supported by the Program for the Promotion of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research of Japan, the National Institute of Biomedical Innovation (NIBIO) of Japan and the Research Grant for Cardiovascular Diseases from the Ministry of Health, Labour and Welfare and the Research Grant of Cardiovascular Diseases (18C-7) from the Ministry of Health, Labour and Welfare.

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DIABETES RESEARCH AND CLINICAL PRACTICE 85 (2009) 132–138

Contents lists available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

International Diabetes Federation

Association study of 11β-hydroxysteroid dehydrogenase type 1 gene polymorphisms and metabolic syndrome in urban Japanese cohort

Yoshihiro Miyamoto [a,*], Hiroko Morisaki [b], Itaru Yamanaka [b], Yoshihiro Kokubo [c], Hiroaki Masuzaki [d], Akira Okayama [c], Hitonobu Tomoike [e], Kazuwa Nakao [d], Tomonori Okamura [c], Yasunao Yoshimasa [a], Takayuki Morisaki [b]

[a] Department of Atherosclerosis and Diabetes, National Cardiovascular Center, Osaka, Japan
[b] Department of Bioscience, National Cardiovascular Center Research Institute, Osaka, Japan
[c] Department of Preventive Cardiology, National Cardiovascular Center, Osaka, Japan
[d] Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan
[e] National Cardiovascular Center Hospital, Osaka, Japan

ARTICLE INFO

Article history:
Received 13 September 2008
Received in revised form
11 May 2009
Accepted 20 May 2009
Published on line 16 June 2009

Keywords:
11β-Hydroxysteroid dehydrogenase type 1
Metabolic syndrome
Single nucleotide polymorphism
Japanese
Haplotype

ABSTRACT

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1), one of the isoforms of the 11β-hydroxysteroid dehydrogenase enzymes, acts as an oxo-reductase to reactivate cortisone to cortisol, plays a critical role in tissue-specific corticosteroid reactions, and is therefore a key molecule associated with the development of metabolic syndrome. We investigated whether variations in the 11β-HSD1 gene correlated with metabolic syndrome. We performed case–control study using a population-based urban Japanese cohort. Among 3005 urban residents, we examined 431 subjects diagnosed with metabolic syndrome according to the Japanese definition and 777 subjects with none of metabolic syndrome criteria as control. We genotyped three single nucleotide polymorphisms (SNPs) (+9410T>A, +17925C>T, +27447G>C) across the 11β-HSD1 gene in them and analyzed the associations of SNPs and haplotypes with metabolic syndrome. The +9410A allele showed a tendency to metabolic syndrome (OR = 1.5, 95%C.I., 1.0–2.2; P = 0.041 and Bonferroni corrected P = 0.123) without statistical significance. However, we could not find any significant association between metabolic syndrome and SNPs in the 11β-HSD1 gene. Our findings indicate that polymorphisms and haplotypes in the 11β-HSD1 gene are not significantly associated with metabolic syndrome in the Japanese population.

1. Introduction

Two isoforms of the 11β-hydroxysteroid dehydrogenase enzyme (11β-HSD), 11β-HSD type 1 (11β-HSD1) and 11β-HSD type 2 (11β-HSD2), catalyze the conversion between hormonally active cortisol and inactive cortisone [1]. 11β-HSD1 acts as an oxo-reductase that reactivates cortisone to cortisol [1] and is an abundant intracellular component in adipose tissue, liver and central nervous system [1–3]. In contrast, 11β-HSD2 is a dehydrogenase that inactivates cortisol to cortisone and is exclusively expressed in organs involved in water and electrolyte metabolism, such as the colon, kidney, sweat

* *Corresponding author.* Tel.: +81 6 6833 5012; fax: +81 6 6872 7486.
E-mail address: miyamoty@hsp.ncvc.go.jp (Y. Miyamoto).
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doi:10.1016/j.diabres.2009.05.017

gland, and placenta [1,4]. This differential expression provides a mechanism for tissue-specific corticosteroid receptor activation that is independent of circulating cortisol concentrations [1,5]. Moreover, studies using animal models have shown that 11β-HSD1 increases intracellular glucocorticoid levels by converting circulating 11-dehydrocorticosterone (cortisone in humans) into active corticosterone (cortisol) through 11β-reductase in adipocytes decrease intracellular glucocorticoid levels [6–9]. In human, 11β-HSD activity in adipose tissue was positively correlated with BMI [10] and 11β-HSD1 inhibition enhances insulin sensitivity and provides a new approach to control metabolic diseases, including type 2 diabetes [11–13].

Epidemiologic studies have indicated that metabolic syndrome has become more prevalent in Western and Asian countries due to both environmental factors and lifestyle changes, such as a high-calorie diet and sedentary behavior. However, there is also evidence that certain individuals are genetically predisposed to metabolic syndrome and its related traits. Polymorphisms in the HSD11B1 gene which encodes 11β-HSD1 have been reported to be associated with type 2 diabetes [14] and hypertension [15]. In particular, Gelernter-Yaniv et al. reported the positive association of the ins4436A SNP in the HSD11B1 gene with BMI and insulin resistance in obese children [16]. However, this association has been inconsistent, probably because of differences in sample size and ethnicity [17].

In light of the possible involvement of 11β-HSD1 in metabolic syndrome, we investigated whether genetic variants of the HSD11B1 gene are associated with metabolic syndrome.

2. Methods

2.1. *Subjects and definition of metabolic syndrome*

We recruited 3655 residents on population-based cohort (Suita, Osaka Prefecture, Japan) from April 2002 to February 2004 and obtained written informed consent to study SNPs. The study design was approved by the Committee on Genetic Analysis and Gene Therapy and the ethics committee of the National Cardiovascular Center. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. Of the 3655 participants, 3005 were included in the study because blood could be collected from them after a 12-h fast and because all three single nucleotide polymorphisms (SNPs) of the HSD11B1 gene in these subjects were successfully genotyped. According to the Japanese consensus determined by eight scientific societies including the Japanese Society of Internal Medicine, metabolic syndrome is defined as central obesity (waist circumference ≥85 cm for men and ≥90 cm for women) plus any two of the following three factors: dyslipidemia (triglycerides >1.69 mmol/l (150 mg/dl) and/or high-density lipoprotein (HDL) cholesterol <1.03 mmol/l (40 mg/dl), or lipid-lowering therapy), hypertension (systolic blood pressure (SBP) ≥130 and/or diastolic blood pressure (DBP) ≥85 mmHg, or antihypertensive therapy), and fasting plasma glucose ≥6.11 mmol/l (110 mg/dl) or previously diagnosed type 2 diabetes [18]. Subjects with none of these metabolic syndrome criteria were defined as controls. Among 3005 persons, 431 persons met the metabolic syndrome criteria, 777 persons did not meet any one of the metabolic syndrome criteria, and 1797 persons who belonged neither to metabolic syndrome nor to controls were indicated as intermediate in Table 1. The Japanese criteria for metabolic syndrome differ from those of the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III), which is considered present when at least three of the five traits including an increased waist circumference, blood pressure elevation, low HDL cholesterol, high triglycerides, and hyperglycemia. As we thought whether a 11βHSD gene was involved in the crises of the metabolic syndrome with the pathology which made visceral fat accumulation a base, we used the Japanese criertia for metabolic syndrome.

2.2. *Clinical parameters*

Blood pressure was measured after at least 10 min of rest in the sitting position. The mean values of two SBP or DBP measurements obtained by a physician using a mercury sphygmomanometer (recorded >3 min apart) were used for analysis. After 12 h of fasting, blood samples were collected, and total cholesterol, HDL-cholesterol, and triglyceride levels were measured with an autoanalyzer (Toshiba TBA-80) in accordance with the Lipid Standardization Program of the US Centers for Disease Control and Prevention through the Osaka Medical Center for Health Science and Promotion, Japan.

2.3. *Anthropometric estimates*

The participants, wearing no shoes and only underwear, were weighed on an electronic scale, and results were recorded to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm using height meter with the subject standing. Waist diameters were measured to the nearest 1.0 cm at the height of the navel upon breath intake using a non-extendable linen tape measure.

2.4. *Screening and identification of SNPs in the human HSD11B1 gene*

Genomic DNA samples were isolated from peripheral leukocytes of the participants. Eight primer sets were designed to amplify the promoter and intron/exon boundaries of the HSD11B1 gene, and an initial SNP screening was performed using 48 randomly chosen DNA samples. Screening for genetic variants was performed using a denaturing HPLC method, in which the PCR products were analyzed using WAVE DNA Fragment Analysis and WAVEMAKER software 4.0 (Transgenomic Inc., Omaha, NE, USA), following the manufacturer's protocol. All detected variations were confirmed by a direct sequencing using an ABI 3700 (Applied Biosystems, Foster City, CA, USA). SNPs were genotyped using TaqMan PCR (ABI PRISM 7900HT, Applied Biosystems). The validity of the detection systems was verified prior to the large-scale study, using 48 samples that were genotyped at the initial screening.

2.5. *Estimation of haplotype frequencies and evaluation of linkage disequilibrium (LD) patterns in the HSD11B1 gene*

We estimated the frequencies of the haplotypes and the coefficient for LD (D′ and r^2 value) among SNPs using Haploview software version 3.32 (http://www.broad.mit.edu/mpg/haploview/).

2.6. *Statistical analysis*

Clinical parameters were compared between metabolic syndrome and control groups using the paired t-test, and a trend analysis for clinical parameters was performed using the Tukey–Kramer HSD test. Fasting blood glucose and triglyceride levels were transformed to natural logarithms before analysis. The association between the risk haplotype and metabolic syndrome was assessed by the chi-square test using Haploview software (http://www.broad.mit.edu/haploview/haploview). This software enables a haplotype population frequency estimation and tests the significant association by Z-test. The genotypic relative risk was assessed by comparing the metabolic syndrome group with the control group and calculating the odds ratio (OR) and the 95% confidence interval (C.I.), using a logistic regression analysis after adjusting for age and sex.

All P values are two-tailed, and P values below 0.05 were considered statistically significant after Bonferroni correction. Statistical analyses were performed using JMP software, version 6.0 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. *Clinical features of metabolic syndrome subjects*

Table 1 shows the clinical characteristics of metabolic syndrome and control subjects. Men had a higher prevalence of metabolic syndrome than women (men, 24.2%; women, 6.1%) and metabolic syndrome subjects were older than subjects without the syndrome (age, 67.6 ± 9.5; 59.5 ± 11.5 years, respectively). Metabolic syndrome had a significantly higher body mass index (BMI), waist circumference, systolic and diastolic blood pressures, fasting glucose, HbA1c, and triglyceride levels and significantly lower HDL-cholesterol, reflecting the criteria of metabolic syndrome. Total cholesterol levels were not significantly different between the groups.

3.2. *Identification of polymorphisms in the HSD11B1 gene*

Forty-eight individuals were examined for polymorphisms in the 11β-HSD1 gene using the WAVE system. A total of seven SNPs and an insertion polymorphism were found in the gene. All eight polymorphisms were in Hardy–Weinberg equilibrium, and the seven SNPs were reported in the NCBI db SNP database (http://www.ncbi.nlm.nih.gov/SNP/). Two of the eight polymorphisms were located in the promoter region (−718T>A, −658G>A), two polymorphisms in intron 3 (+1930insA, +1972T>G), three SNPs in intron 4 (+9410T>A, +17925C>T, +27447G>C) and one SNP in the 3′UTR (+29813G>A) (Table 2).

We evaluated the linkage disequilibrium pattern among these eight polymorphisms and defined haplotypes using DNA from 48 persons. As shown in Fig. 1, one LD block consisted of SNPs from SNP-3 to SNP-8. As the D′ between SNP-7 and SNP-3, -4, and -8 were all 1.00, respectively, and the r^2 between SNP-7 and SNP-3, -4, and -8 were 1.00, 1.00, and 0.899, respectively, we considered that SNP-7 captured SNP-3, -4, and -8. Taking together with their low allele frequencies of SNP-1 and -2, we used three SNPs (SNP-5, -6, and -7) for determining a haplotype of the LD block and this association study.

3.3. *Association of SNPs and haplotypes of the HSD11B1 gene with metabolic syndrome*

As shown in Table 3, the +9410T>A SNP was nominally associated with metabolic syndrome after adjusting for sex

Table 1 – Comparison of clinical parameters among metabolic syndrome, intermediate and control groups in an urban Japanese population (*n* = 3005).

	Control (*n* = 777)	Intermediate (*n* = 1797)	MS (*n* = 431)	P*
Men (*n*, %)	198, 25.5	841, 46.8	331, 76.8	<0.001
Age (year)	59.5 ± 11.5	68.0 ± 10.0	67.6 ± 9.5	<0.001
BMI (kg/m^2)†	20.8 ± 2.3	23.0 ± 2.9	25.9 ± 2.7	<0.001
Waist (cm)†	77.3 ± 6.3	85.0 ± 8.0	93.1 ± 6.0	<0.001
SBP (mmHg)†	112.1 ± 9.9	135.2 ± 18.2	140.9 ± 16.0	<0.001
DBP (mmHg)†	71.0 ± 7.5	79.3 ± 9.4	83.6 ± 9.7	<0.001
FBG (mmol/l)†	5.02 ± 0.42	5.52 ± 1.04	6.59 ± 1.76	<0.001
HbA1c (%)†	5.2 ± 0.3	5.5 ± 0.7	6.1 ± 1.1	<0.001
T-Cho (mmol/l)	5.35 ± 0.83	5.40 ± 0.82	5.35 ± 0.92	1.000
TG (mmol/l)†	0.83 ± 0.30	1.19 ± 0.68	1.89 ± 1.00	<0.001
HDLc (mmol/l)†	1.75 ± 0.39	1.55 ± 0.39	1.28 ± 0.33	<0.001

MS: metabolic syndrome, SBP: systolic blood pressure, DBP: diastolic blood pressure, FBG: fasting blood glucose, TG: triglycerides, T-Cho: total cholesterol, HDLc: HDL cholesterol. Data are shown as the mean ± SD. The laboratory data reported in milligram per deciliter can be converted to SI units as follows: total cholesterol, HDL cholesterol: mg/dl × 0.02586 = mmol/l; triglycerides: mg/dl × 0.01129 = mmol/l; fasting blood glucose: mg/dl × 0.05556 = mmol/l.

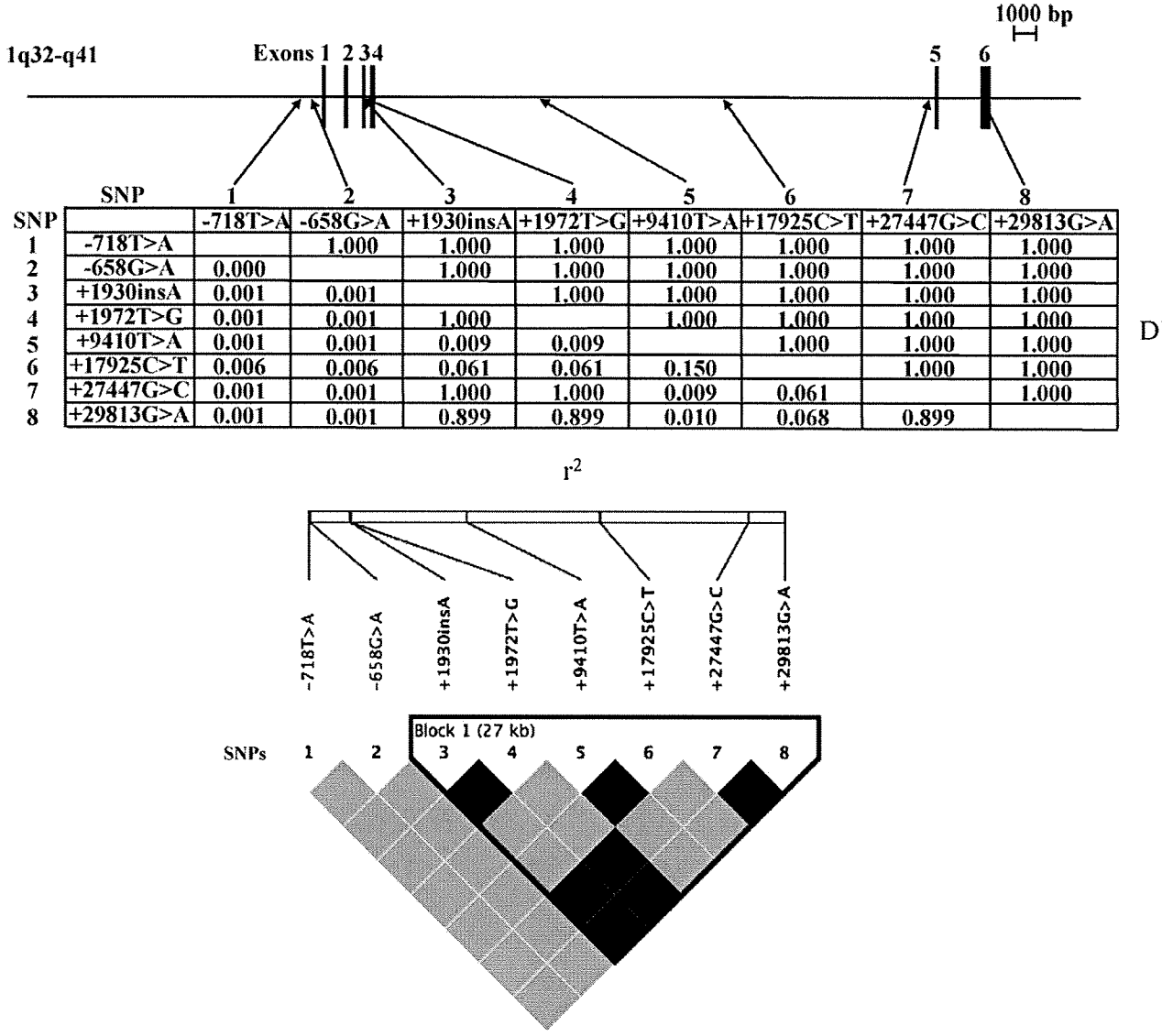
* P-values for the comparison between MS and control groups.

† P-values for the trend among the three groups were less than 0.05.

Table 2 – Characteristics of the polymorphisms in the 11β-HSD1 gene locus.

SNP	Position genome[a]	dbSNP ID	Variation	Location	Frequency of minor alleleb[b]
1	−718	rs860185	T>A	5′flanking	0.010
2	−658		G>A	5′flanking	0.010
3	+1930		insA	Intron 3	0.104
4	+1972	rs12086634	T>G	Intron 3	0.104
5	+9410	rs2236905	T>A	Intron 4	0.073
6	+17925	rs2298930	C>T	Intron 4	0.344
7	+27447	rs932335	G>C	Intron 4	0.104
8	+29813	rs6752	G>A	Exon 6	0.115

[a] Numbers indicate locations relative to A of the ATG translation initiation codon.
[b] Based on screening results of 48 pilot samples.



SNP		-718T>A	-658G>A	+1930insA	+1972T>G	+9410T>A	+17925C>T	+27447G>C	+29813G>A
1	-718T>A		1.000	1.000	1.000	1.000	1.000	1.000	1.000
2	-658G>A	0.000		1.000	1.000	1.000	1.000	1.000	1.000
3	+1930insA	0.001	0.001		1.000	1.000	1.000	1.000	1.000
4	+1972T>G	0.001	0.001	1.000		1.000	1.000	1.000	1.000
5	+9410T>A	0.001	0.001	0.009	0.009		1.000	1.000	1.000
6	+17925C>T	0.006	0.006	0.061	0.061	0.150		1.000	1.000
7	+27447G>C	0.001	0.001	1.000	1.000	0.009	0.061		1.000
8	+29813G>A	0.001	0.001	0.899	0.899	0.010	0.068	0.899	

Fig. 1 – Pairwise linkage disequilibrium in 11β-HSD1 gene evaluated by D′ and r^2. Using the eight SNPs (−718T>A, −658G>A, +1930insA, +1972T>G, +9410T>A, +17925C>T, +27447G>C, +29813G>A) in the 11β-HSD1 gene, we calculated pairwise r^2 and D′ for each SNP pair and evaluated the linkage disequilibrium pattern of the 11β-HSD1 gene in 48 pilot samples. The three SNPs (+1930insA, +1972T>G and +27447G>C) are completely linked each other as both r^2 and D′ among these SNPs equal to 1.0. A bold line surrounds a haplotype block.

Table 3 – Logistic analysis of 11β-HSD1 SNPs with the risk of metabolic syndrome adjusted by age and sex (both) or by age (men or women).

	MS n/n' (%)	Control n/n' (%)	OR (95%C.I.)	P	Pcorr
Both					
+9410T>A	61/370 (14.2)	91/686 (11.7)	1.5 (1.0–2.2)	0.041	NS
+17925C>T	249/182 (57.8)	453/324 (58.3)	1.0 (0.7–1.2)	0.683	NS
+27447G>C	118/313 (27.4)	199/578 (25.6)	1.1 (0.8–1.4)	0.632	NS
Men					
+9410T>A	45/286 (13.6)	16/182 (8.1)	1.9 (1.1–3.5)	0.029	NS
Women					
+9410T>A	16/84 (16.0)	75/504 (13.0)	1.1 (0.6–2.1)	0.683	NS

MS: metabolic syndrome, n: number of minor homozygote and heterozygote, n': number of major homozygote, %: $n/(n+n') \times 100$, 95%C.I.: 95% confidence interval.
Odds ratio and 95%C.I. is expressed as per copy of the minor allele for additive model.
Pcorr is *P* values after Bonferroni correction.
NS means not significance.

and age (OR = 1.5 for allelic effect, 95%C.I., 1.0–2.2; P = 0.041 and Bonferroni corrected P = 0.123). In only men, +9410T>A SNP was nominally associated with metabolic syndrome, while the higher frequency of metabolic syndrome in men lead to the higher power in comparison with women. Taken together all, after considering multiple comparisons we could not find any statistically significant association between metabolic syndrome and SNPs in the HSD11B1 gene. Furthermore, we could not find any significant association between metabolic syndrome of the ATP III criteria and these three SNPs, respectively. We next performed the covariance analysis of the traits related to metabolic syndrome including BMI, waist circumference, systolic and diastolic blood pressures, fasting glucose, HbA1c, and triglyceride and HDL-cholesterol levels in person with or without the +9410T>A SNP, but the SNP did not affect these clinical parameters in total population or only men (Table 4).

Next we studied haplotypes of the HSD11B1. The association between haplotypes comprising SNP-5, -6, and -7 and metabolic syndrome revealed that any haplotype could not have a significant susceptibility to metabolic syndrome (Table 5). The ATG haplotype was nominally associated with a increased risk of metabolic syndrome in men (metabolic syndrome 7.1%, control 4.0%; OR = 1.82, 95%C.I., 1.01–3.25; P = 0.042 and Bonferroni corrected P = 0.168), while the TTG haplotype was nominally associated with a decreased risk of metabolic syndrome in men (metabolic syndrome 26.4%, control 32.6%; OR = 0.74, 95%C.I., 0.57–0.98; P = 0.033 and Bonferroni corrected P = 0.132). Although only the TTG haplotype had decreased risk effect among haplotypes with +9410T, the association of these haplotypes with metabolic syndrome is mainly explained by +9410T>A SNP.

4. Discussion

This study was a case–control study using a population-based urban Japanese cohort. Metabolic syndrome was diagnosed according to the 2005 Japanese definition [18] and the control subjects were defined as having none of the components of this syndrome. Using these criteria, we obtained 431 individuals with metabolic syndrome and 777 control subjects for

Table 4 – Comparison of clinical parameters in urban Japanese men and women (n = 3005) according to 11β-HSD1 gene +9410T>A genotype.

	Men (n = 1370)			Women (n = 1635)		
	TT (n = 1180)	TA + AA (n = 189)	P	TT (n = 1400)	TA + AA (n = 235)	P
BMI (kg/m^2)	24.1 ± 0.1	23.0 ± 2.9	0.502	22.9 ± 0.1	23.0 ± 0.2	0.622
Waist (cm)	87.7 ± 0.2	87.5 ± 0.6	0.800	84.4 ± 0.3	84.1 ± 0.6	0.695
SBP (mmHg)	132.6 ± 0.6	133.8 ± 1.5	0.442	129.8 ± 0.5	129.3 ± 1.3	0.686
DBP (mmHg)	79.9 ± 0.3	80.4 ± 0.8	0.587	77.1 ± 0.3	77.2 ± 0.7	0.834
FBG (mmol/l)	5.83 ± 0.04	5.96 ± 0.11	0.256	5.47 ± 0.03	5.55 ± 0.07	0.309
HbA1c (%)	5.61 ± 0.03	5.70 ± 0.07	0.233	5.45 ± 0.02	5.47 ± 0.05	0.607
TG (mmol/l)	1.47 ± 0.03	1.44 ± 0.07	0.732	1.12 ± 0.02	1.15 ± 0.04	0.582
HDLc (mmol/l)	1.35 ± 0.01	1.38 ± 0.03	0.369	1.64 ± 0.01	1.62 ± 0.03	0.418

SBP: systolic blood pressure, DBP: diastolic blood pressure, FBG: fasting blood glucose, TG: triglycerides, HDLc: HDL cholesterol. Data are shown as the adjusted mean ± SE. These values were obtained after adjusting for age by the least squares method. The laboratory data reported in milligram per deciliter can be converted to SI units as follows: total cholesterol, HDL cholesterol: mg/dl × 0.02586 = mmol/l; triglycerides: mg/dl × 0.01129 = mmol/l; fasting blood glucose: mg/dl × 0.05556 = mmol/l.
P-values for the comparison between TT and TA + AA genotype groups.

Table 5 – Frequency of 11β-HSD1 gene haplotypes constructed by SNPs + 9410T>A, +17925C>T, and +27447G>C and their association with metabolic syndrome.

Gender	Haplotype	Total	MS	Control	χ^2	OR	95%C.I.	P	Pcorr
Both	TCG	0.515	0.514	0.510	0.040	1.02	0.86–1.20	0.841	NS
	TTG	0.277	0.266	0.290	1.653	0.88	0.73–1.07	0.199	NS
	TCC	0.133	0.147	0.140	0.225	1.06	0.84–1.34	0.635	NS
	ATG	0.074	0.073	0.060	1.609	1.24	0.89–1.73	0.205	NS
Men	TCG	0.509	0.526	0.477	2.323	1.21	0.95–1.56	0.128	NS
	TTG	0.280	0.264	0.326	4.563	0.74	0.57–0.98	0.033	NS
	TCC	0.138	0.139	0.157	0.617	0.87	0.61–1.23	0.432	NS
	ATG	0.073	0.071	0.040	4.141	1.82	1.01–3.25	0.042	NS
Women	TCG	0.521	0.475	0.521	1.427	0.83	0.62–1.12	0.232	NS
	TTG	0.275	0.270	0.278	0.055	0.96	0.69–1.35	0.814	NS
	TCC	0.129	0.175	0.135	2.290	1.36	0.91–2.04	0.130	NS
	ATG	0.075	0.075	0.066	0.488	1.22	0.70–2.14	0.485	NS

MS: metabolic syndrome, 95%C.I.: 95% confidence interval.
Pcorr is *P* values after Bonferroni correction.
NS means not significance.

the case–control study. We could not find any significant association between SNPs in the HSD11B1 gene and metabolic syndrome.

11β-HSD1 is expressed abundantly in adipose tissue and reactivates cortisone to cortisol [1]. Recent experiments using transgenic and knockout mice suggest that 11β-HSD1 plays a critical role in metabolic deterioration [6–9]. When cortisol generation is increased in peripheral tissues, the overall cortisol reaction is also increased. In humans, 11β-HSD1 expression is heightened in the adipose tissue of obese individuals [10]. Therefore, 11β-HSD1 is a promising target for the pharmacological inhibition in metabolic syndrome patients [11–13].

A previous study showed that a HSD11B1 gene polymorphism is associated with BMI and insulin resistance in a group of obese Pima Indian children [16] with type 2 diabetes mellitus and hypertension and reported that 11β-HSD1 mRNA concentrations were associated with adiposity [14,15]. The T → G polymorphism in the 3rd intron (rs12086634) protects against diabetes in Pima Indians [14] and reduces 11β-HSD1 gene transcription in vitro [19], which is consistent with reduced cortisol generation within cells. The rs12086634 polymorphism was completely in linkage disequilibrium with the rs932335 SNP (+27447G>C) that was analyzed in this study. We did not find a positive association between the +27447G>C SNP and metabolic syndrome in Japanese men.

There are some limitations in this study. First limitation was the Japanese criteria for metabolic syndrome, which is different from the ATP III criteria. Third, there were deviations in social factors, including age, gender, race/ethnicity, geographic location, and socioeconomic status. This cohort consisted of urban citizens residing in a subtropical area with a temperate climate. Most subjects were Asian with a higher percentage of elderly people.

In summary, the HSD11B1 gene is not associated with metabolic syndrome in Japanese. However, taken together with previous results, 11β-HSD1 might be involved in metabolic syndrome pathogenesis by modulating lipid metabolism and gluconeogenesis. Further studies are needed to investigate the role of 11β-HSD1 in metabolic syndrome.

Conflict of interest

The authors state that they have no conflict of interest.

Acknowledgments

We are grateful to the following people for their support in our population survey: Dr. Yasushi Kotani, President; Dr. Katsuyuki Kawanishu, the Co-President; other members of the Suita City Medical Association; and Mr. Kinzo Harada, Director of the City Health Center. We also thank the members of our participants' group (Satsuki-Junyu-kai) for their cooperation with and support of our survey of risk factors and prevention of cardiovascular disease. We also thank Dr. Soichiro Kitamura, President of the National Cardiovascular Center, for encouraging our work. This study was supported by the Program for the Promotion of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research of Japan, the Research Grant from Special Coordination Funds for Promoting Science and Technology (JST) and the National Institute of Biomedical Innovation (NIBIO) of Japan and the Research Grant for Cardiovascular Diseases from the Ministry of Health, Labour and Welfare of Japan.

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Atherosclerosis 209 (2010) 290–294

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Triglycerides and non-high-density lipoprotein cholesterol and the incidence of cardiovascular disease in an urban Japanese cohort: The Suita study

Tomonori Okamura[a,*], Yoshihiro Kokubo[a], Makoto Watanabe[a], Aya Higashiyama[a], Yuu Ono[a], Yoshihiro Miyamoto[b], Yasunao Yoshimasa[b], Akira Okayama[c]

[a] Department of Preventive Cardiology, National Cardiovascular Center, Osaka, Japan
[b] Department of Atherosclerosis and Diabetes, National Cardiovascular Center, Osaka, Japan
[c] The First Institute for Health Promotion and Health Care, Japan Anti-tuberculosis Association, Tokyo, Japan

ARTICLE INFO

Article history:
Received 15 July 2009
Received in revised form 18 August 2009
Accepted 3 September 2009
Available online 12 September 2009

Keywords:
Triglycerides
Myocardial infarction
Stroke
Cohort studies
Lipids and lipoprotein

ABSTRACT

Objective: The impact of elevated triglycerides (TG) and non-high density lipoprotein cholesterol (non-HDLC) on the incidence of stroke and myocardial infarction (MI) has not been well evaluated in Asian populations such as in Japan, which have a lower incidence of myocardial infarction, but a higher risk of stroke than Western populations.

Methods: The authors conducted an 11.7-year prospective study ending in 2005 of 5098 Japanese aged 30–79 living in an urban population, initially free of stroke or MI. The relationship between serum lipids and the risk for stroke and MI was determined by dividing the participants into four groups stratified by the combination of serum levels of TG and non-HDLC. The cut-off value was 1.7 mmol/L for TG and 4.9 mmol/L for non-HDLC.

Results and conclusion: The total person-years were 59,774 (27,461 for men and 32,313 for women). During the follow-up period, there were 113 cases of MI and 180 of stoke (with 116 cerebral infarctions). Compared with the low TG/low non-HDLC group, the hazard ratio (95% confidence interval) for MI in the high TG/high non-HDLC group was 2.55 (1.53–4.24) after adjustment for other cardiovascular risk factors. The hazard ratio for cerebral infarction in the high TG alone group was 1.63 (1.03–2.56); however, the risk of cerebral infarction was not significantly increased in the other groups. High serum levels of TG and non-HDLC are both important targets for the prevention of cardiovascular disease in Japan.

1. Introduction

Previous studies suggested that high levels of serum total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) are causal risk factors for coronary artery disease (CAD) [1–4] and possibly for ischemic stroke [5]. However, less attention has been paid to high serum levels of triglycerides (TG) [6–8]. Furthermore, although the US National Cholesterol Education Program Adult Treatment Panel guideline III (NCEP-ATP III) has set goals for non-high-density lipoprotein cholesterol (non-HDLC) after the achievement of LDL-C goals in patients with elevated TG [9], the impact of TG and non-HDLC on the incidence of cardiovascular disease (CVD) has not been evaluated in the Japanese population, which has a lower incidence of CAD but a higher risk of stroke than Western populations [10].

Therefore, our a priori hypothesis was that the coexistence of high serum TG and non-HDLC increases the risk of CAD and stroke in the Japanese population. To investigate this hypothesis, we performed a long-term prospective study in an urban, community-dwelling Japanese population.

2. Methods

2.1. Populations

The Suita study, a cohort study for CVD of urban residents was established in 1989. The details of this study have been described elsewhere [4,11–14]. Briefly, 6485 men and women aged 30–79 years had a baseline survey at the National Cardiovascular Center between September 1989 and March 1994. Of these, a total of 1387 were excluded for the following reasons: past history of coronary heart disease or stroke ($n=210$), lack of participation in the baseline survey ($n=79$), non-fasting visit ($n=166$), use of lipid-lowering agents ($n=125$), missing data ($n=109$), and lost to follow-up ($n=698$). Data from the remaining 5098 participants (2404 men and 2694 women) were included in the analysis. This

* Corresponding author at: Department of Preventive Cardiology, National Cardiovascular Center, 5-7-1, Fujishiro-dai, Suita, Osaka 565-8565, Japan. Tel.: +81 6 6833 5012x2228/2188; fax: +81 6 6833 5300.
E-mail address: okamurat@hsp.ncvc.go.jp (T. Okamura).

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doi:10.1016/j.atherosclerosis.2009.09.012

cohort study was approved by the Institutional Review Board of the National Cardiovascular Center.

2.2. Baseline examination

Blood samples were collected after the participants had fasted for at least 10 h. The samples were centrifuged immediately and a routine blood examination was performed that included serum total cholesterol (TC), HDL cholesterol, TG and glucose levels.

Blood pressure was measured in triplicate on the right arm after 5 min of rest by well-trained physicians using a standard mercury sphygmomanometer. The average of the second and third measurements was used for analysis. Hypertension was defined as either a systolic blood pressure (SBP) ≥ 140 mmHg, a diastolic blood pressure (DBP) ≥ 90 mmHg or the use of antihypertensive agents. Diabetes was defined as a fasting serum glucose ≥ 7.0 mmol/L (126 mg/dL), the use of anti-diabetic agents, or both. Height with bare feet and weight in light clothing were measured. Waist circumference (WC) was measured at umbilical level in a standing position. Metabolic syndrome (MetS) was defined using modified NCEP-ATP III criteria [13], of which abdominal obesity was defined according to the International Obesity Task Force central obesity criteria for Asia [15].

Public health nurses obtained information on the smoking, drinking and medical histories.

2.3. Endpoint determination

The endpoint determination was previously reported [4,11–14]. The endpoints of the present study were: (1) the first myocardial infarction (MI) or stroke event; (2) death; (3) leaving Suita city; or (4) December 31, 2005.

The first step in the survey for MI and stroke involved checking the health status of all participants by repeated clinical visits every two years and yearly questionnaires by mail or telephone. In the second step, in-hospital medical records of participants who were suspected of having an MI or stroke were reviewed by registered hospital physicians or research physicians, who were blinded to the baseline information. The criteria for stroke were defined according to the US National Survey of Stroke criteria [16]. For each stroke subtype [i.e., cerebral infarction, intracerebral hemorrhage, and subarachnoid hemorrhage], a definite diagnosis was established based on the computed tomography, magnetic resonance imaging, or autopsy. The criteria for definite and probable MI were defined according to the criteria of the MONICA (Monitoring Trends and Determinants of Cardiovascular Disease) project [17]. Sudden deaths of unknown origin that occurred within 24 h of the onset were classified as MI in the present study.

2.4. Statistical analysis

The relationship between serum lipids and the risk of MI and stroke was described by dividing the participants into four groups stratified by the combination of serum levels of TG and non-HDLC. We used 1.7 mmol/L (150 mg/dL) of serum TG as a cut-off point for high serum TG according to the classification of NCEP-ATP III [9] and that of the Japan Atherosclerosis Society [3]. The category of non-HDLC ≥4.9 mmol/L (190 mg/dL) was defined as a high serum non-HDLC, which was equivalent to 6.2 mmol/L (240 mg/dL) of TC or 4.1 mmol/L (160 mg/dL) of LDL-C, because non-HDLC was usually 0.8 mmol/L (30 mg/dL) higher than LDL-C [9,18–19].

Continuous variables between groups were compared by analysis of variance and categorical variables were compared by a chi-square test. The hazard ratio (HR) for MI or stroke was calculated using a proportional hazards model adjusted for age, hypertension (dichotomous variable), diabetes, HDL-C, body mass index (BMI), smoking (never-smoked; ex-smoker; current smoker) and drinking (never-drank; ex-drinker; regular drinker) (model 1). Sex-combined analysis with further adjustment for sex was also performed. Another statistical model after replacement of BMI and hypertension with WC and SBP level (continuous variable) was also performed (model 2).

All confidence intervals were estimated at the 95% level and significance was set at a P value of <0.05. The Statistical Package for the Social Sciences (SPSS Japan Inc. version 15.0J, Tokyo, Japan) was used for all the analyses.

3. Results

The median and interquartile range of serum TG in the baseline survey was 1.29 mmol/L (0.90, 1.90) in men and 0.98 mmol/L (0.73, 1.41) in women. The mean baseline serum non-HDLC was 3.93 ± 0.91 mmol/L in men and 4.03 ± 1.03 mmol/L in women.

The means or prevalence of major cardiovascular risk factors in each group stratified by the combination of serum levels of TG and non-HDLC are summarized in Table 1. There was no significant difference in mean age and the prevalence of smoking among the TG and non-HDLC groups for men. There were significant differences in all other variables. Mean BMI, waist circumference and the prevalence of hypertension and diabetes were highest in the high-TG/high non-HDLC group, whereas the values of these parameters were lowest in the low-TG/low-non-HDLC group for both sexes. The prevalence of Mets was much higher in the high-TG groups than in the low-TG groups irrespective of non-HDLC level.

The total person-years were 59,774 (27,461 for men and 32,313 for women), with a mean follow-up period of 11.7 years. During the follow-up period, there were 113 first MIs and 180 first strokes. The strokes consisted of 28 intracerebral hemorrhages, 116 cerebral infarctions, 21 subarachnoid hemorrhages and 15 unclassified cases.

Table 2 shows the number of cases, age and multivariable-adjusted HRs for MI stratified by TG and non-HDLC. Compared with the low TG/low non-HDLC group, the HR for MI in the high TG/high non-HDLC group was 2.05 (95% confidence interval, CI, 1.08–3.90) in men, 3.79 (95% CI, 1.58–9.14) in women and 2.55 (95% CI, 1.53–4.24) in both sexes combined in multivariable adjusted model 1. We did not observe a significant increase in the HR for MI in the other groups. Similar results were observed after replacement of BMI and hypertension with WC and SBP level (model 2).

Table 3 shows the multivariable-adjusted HRs for cerebral infarction stratified by levels of TG and non-HDLC. Compared with the low TG/low non-HDLC group, the HR for cerebral infarction in the high TG alone group (high TG/low non-HDLC group) was 1.45 (95% CI, 0.84–2.50) in men, 2.09 (95% CI, 0.92–4.73) in women and 1.63 (95% CI, 1.03–2.56) in both sexes combined in statistical model 1. There was no significant increase of cerebral infarction in the other groups. Similar results were also observed in statistical model 2.

The incidence of total stroke, intracerebral hemorrhage and subarachnoid hemorrhage was not related to TG and non-HDLC levels in either sex. When the participants were divided into two groups by age (<60 and ≥60), the results of all the analyses listed above were similar in both age groups (data not shown).

4. Discussion

To our knowledge, this is the first cohort study in Japan to clarify the risk for MI and ischemic stroke of high serum level of TG, non-HDLC and both. The risk for MI of both high serum TG and non-HDLC was considerably higher than the risk without both or with only one. This relationship was similarly observed in both men and

Table 1
Means and prevalence of major cardiovascular risk factors in each group stratified by the combination of serum levels of triglycerides (TG) and non-high-density lipoprotein cholesterol (non-HDLC).

Variables	Low TG/low Non-HDLC		Low TG/high Non-HDLC		High TG/low Non-HDLC		High TG/high Non-HDLC		*P* value
Men									
No. of subjects	1532		117		550		205		
Non-HDLC (stratum mean), mmol/L	3.6	(0.7)	5.4	0.4	4.0	0.6	5.5	0.5	
Triglycerides (stratum median), mmol/L	1.0	(0.8, 1.3)*	1.3	(1.0, 1.5)*	2.2	(1.9, 2.9)*	2.4	(2.0, 3.7)*	
Age, years	55.8	(13.5)	57.4	(12.9)	54.8	(12.7)	54.8	(11.8)	0.16
HDLC, mmol/L	1.4	(0.3)	1.3	(0.3)	1.1	(0.3)	1.1	(0.2)	<0.01
BMI, kg/m^2	22.2	(2.8)	23.1	(3.1)	23.8	(2.6)	24.2	(2.6)	<0.01
Waist circumference, cm	80.8	(7.9)	82.7	(8.6)	85.7	(7.0)	86.3	(6.9)	<0.01
Systolic blood pressure, mmHg	127	(21)	129	(20)	130	(20)	132	(21)	<0.01
Diastolic blood pressure, mmHg	78	(12)	79	(12)	81	(11)	82	(11)	<0.01
Hypertension, %	30.0		35.0		36.4		38.0		0.01
Diabetes, %	4.8		4.3		7.5		9.3		0.02
Metabolic syndrome, %**	4.5		4.3		45.1		47.8		<0.01
Smoking, %									
Current smoker	49.9		43.6		53.5		47.3		0.51
Ex-smoker	30.3		35.0		28.4		32.7		
Never-smoker	19.8		21.4		18.2		20.0		
Drinking, %									
Current drinker	76.0		63.2		76.4		69.3		0.02
Ex-drinker	3.6		6.0		2.9		5.4		
Never-drinker	20.4		30.8		20.7		25.4		
Women									
No. of subjects	1956		290		256		192		
Non-HDLC (stratum mean), mmol/L	3.6	(0.7)	5.5	(0.5)	4.2	(0.5)	5.8	(0.8)	
Triglycerides (stratum median), mmol/L	0.9	(0.7, 1.1)*	1.2	(0.9, 1.4)*	2.0	(1.8, 2.4)*	2.4	(2.0, 3.0)*	
Age, years	51.5	(12.9)	59.3	(9.6)	57.9	(11.2)	60.7	(8.8)	<0.01
HDLC, mmol/L	1.5	(0.3)	1.4	(0.3)	1.2	(0.3)	1.1	(0.3)	<0.01
BMI, kg./m2	21.7	(3.1)	22.9	(3.1)	23.6	(3.3)	24.2	(3.1)	<0.01
Waist circumference, cm	75.5	(9.8)	79.8	(9.7)	82.7	(10.0)	83.5	(9.7)	<0.01
Systolic blood pressure, mmHg	121	(21)	131	(21)	132	(21)	137	(21)	<0.01
Diastolic blood pressure, mmHg	73	(12)	79	(12)	79	(12)	80	(13)	<0.01
Hypertension, %	20.4		37.9		37.1		48.4		<0.01
Diabetes, %	2.4		4.5		6.6		7.8		<0.01
Metabolic syndrome, %**	7.5		19.3		66.8		74.5		<0.01
Smoking, %									
Current smoker	11.8		8.6		14.5		16.1		0.04
EX-smoker	3.5		2.8		2.7		6.3		
Never-smoker	84.7		88.6		82.8		77.6		
Drinking, %									
Current drinker	34.9		29.3		28.5		24.5		<0.01
Ex-drinker	1.8		0.3		0.8		4.2		
Never-drinker	63.3		70.3		70.7		71.4		

TG, triglycerides; non-HDLC, non-high-density lipoprotein cholesterol; BMI, body mass index. Brackets indicate standard deviation.
Analysis of variance was used for comparisons of multiple group means and the chi-square test was used to compare proportions.

* Inter-quartile range.

** MetS was defined using modified NCEP-ATP III. Abdominal obesity was defined as a waist circumference ≥0.90 m in men and ≥ 0.80 m in women. High blood pressure was defined as average systolic/diastolic blood pressures of ≥ 130/85 mm Hg and/or current medication for hypertension. High triglyceride was defined as serum triglycerides of ≥1.7 mmol/L. Low HDL cholesterol was defined as serum HDL cholesterol levels of <1.03 mmol/L in men and of <1.29 mmol/L in women. High blood glucose was defined as fasting blood glucose of ≥6.1 mmol/L and/or current use of anti-diabetic medication. MetS was defined as the presence of three or more of these components.

women. In contrast, the risk for ischemic stroke was highest in the participants with high TG alone.

TG-rich lipoproteins have been shown to be atherogenic, and thus, they are associated with coronary atherosclerosis [9,19–20]. As NCEP-ATP III pointed out [9], elevated non-HDLC is a good therapeutic target in patients with high TG, because the serum concentration of non-HDLC reflects not only LDL-C but also the cholesterol content of all other TG-rich and apolipoprotein B containing lipoproteins, such as very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), small dense LDL particles and their remnant lipoproteins [19–20]. In the Helsinki Heart study [21], most of the risk for coronary heart disease (CHD) was confined to participants with high levels of both TG and LDL-C. In the West of Scotland Coronary Prevention Study [22], a higher incidence of CHD was observed in men in both the pravastatin and placebo groups when TG was at or above the median level. Pischon et al. suggested that TG added significant information to non-HDLC for CAD risk prediction in a nested case-control study [23]. Our findings are consistent with previous studies.

Similar to previous studies in Japan [4,10], we found no association between non-HDLC and cerebral infarction even in the presence of high serum TG, which may be due to a lower prevalence of atherothrombotic infarction than in Western populations. The ARIC study indicated that TC was associated with increased risk of non-lacunar, non-embolic stroke (atherothrombotic infarction), but not with lacunar or embolic stroke [24]. A recent report from a Japanese rural population showed that LDL-C is a risk factor for only atherothrombotic infraction [25]. Unfortunately, due to the relatively small stroke cases in our study, we were not able to demonstrate an association between any subtype of cerebral infarction and non-HDLC.

It is not clear why participants with high TG alone showed the increased risk for cerebral infarction in the present study. In a meta-analysis of 26 cohort studies in Asia-Pacific area, partici-

Table 2
Age and multivariable-adjusted hazard ratios (95% confidence intervals) for myocardial infarction stratified by TG and non-HDLC groups in an 11.7-year prospective study of 5098 Japanese men and women.

	Low TG/low Non-HDLC	Low TG/high Non-HDLC	High TG/low Non-HDLC	High TG/high Non-HDLC
Men				
Person-years	17410	1288	6358	2404
Case, *n*	45	6	11	14
Age adjusted	1.00	1.63 (0.70-3.83)	0.76 (0.39-1.48)	2.74(1.50-5.02)
Model 1[a]	1.00	1.48 (0.62-3.49)	0.63 (0.32-1.26)	2.05(1.08-3.90)
Model 2[b]	1.00	1.55 (0.66-3.66)	0.64 (0.32-1.29)	2.10 (1.10-3.98)
Women				
Person-years	23652	3455	2936	2270
Case, *n*	14	5	6	12
Age adjusted	1.00	1.59 (0.57-4.40)	2.28 (0.88-5.94)	4.88 (2.25-10.6)
Model 1[a]	1.00	1.63 (0.58-4.26)	1.99 (0.71-5.57)	3.79 (1.58-9.14)
Model 2[b]	1.00	1.55 (0.55-4.38)	1.92 (0.69-5.34)	3.18 (1.34-7.52)
Men and women				
Person-years	41062	4743	9294	4674
Case, *n*	59	11	17	26
Age adjusted	1.00	1.51 (0.79-2.89)	1.04 (0.60-1.78)	3.42 (2.15-5.44)
Model 1[a]	1.00	1.42 (0.74-2.74)	0.86 (0.49-1.53)	2.55 (1.53-4.24)
Model 2[b]	1.00	1.45 (0.75-2.79)	0.87 (0.49-1.54)	2.48 (1.49-4.10)

TG, triglycerides; non-HDLC, non high-density lipoprotein cholesterol.
[a] Multivariable adjusted for age, body mass index, hypertension, diabetes, HDL (high-density lipoprotein) cholesterol, cigarette smoking and alcohol intake by a Cox proportional hazard model. Sex was also adjusted in the men and women combined model.
[b] Replacement of body mass index and hypertension as covariates in model 1 with waist circumference and systolic blood pressure level.

pants grouped in the highest fifth of serum TG had a 50% increased risk of stroke compared with those in the lowest fifth [26]. Recent reviews have also concluded that hypertriglyceridemia seems to be a causal risk factor for ischemic stroke [7–8]. However, abovementioned findings were not able to explain the low incidence of cerebral infarction in the high TG/high non-HDLC group in the present study. An elevated risk for MI might mask the relationship between TG and cerebral infarction; because there would be no further follow-up after a first MI. Another large study concerning about the relationship between serum TG and stroke should be needed.

Recently, we have reported that high serum LDLC and non-HDLC are both associated with an increased risk of MI; and the predictive value of non-HDLC for MI is almost similar to that of LDL-C [4]. However, we did not use serum TG as a covariate to avoid overadjustment, because difference between serum level of LDLC and non-HDLC was automatically determined by serum TG level when serum LDLC value was calculated by the Friedewald formula [27]. Considering all the findings together, non-HDLC and TG may be recommended as beneficial screening markers for primary prevention of CAD in the Japanese community, as they are less expensive and more convenient because non-HDLC can be calculated irrespective of serum TG level.

The present study has some limitations. First, the single TG and non-HDLC measurement at the baseline survey may have underestimated the relationship between these lipids and cardiovascular disease due to regression dilution bias. Furthermore, we did not evaluate longitudinal trend for each risk factor and its medication status after baseline survey. Especially, hypertriglyceridemia is associated with not only present existence of metabolic components, such as hypertension and diabetes, but also new onset

Table 3
Age and multivariable-adjusted hazard ratios (95% confidence intervals) for cerebral infarction stratified by TG and non-HDLC groups in an 11.7-year prospective study of 5098 Japanese men and women.

	Low TG/low Non-HDLC	Low TG/high Non-HDLC	High TG/low Non-HDLC	High TG/high Non-HDLC
Men				
Person-years	17410	1288	6358	2404
Case, *n*	46	2	22	5
Age adjusted	1.00	0.53 (0.13-2.19)	1.51 (0.91-2.52)	0.99 (0.39-2.51)
Model 1[a]	1.00	0.54 (0.13-2.25)	1.45 (0.84-2.50)	0.92 (0.35-2.38)
Model 2[b]	1.00	0.56 (0.14-2.31)	1.48 (0.86-2.56)	0.75 (0.26-2.14)
Women				
Person-years	23652	3455	2936	2270
Case, *n*	20	8	10	3
Age adjusted	1.00	1.77 (0.78-4.02)	2.62 (1.23-5.60)	0.81 (0.24-2.72)
Model 1[a]	1.00	1.52 (0.66-3.50)	2.09 (0.92-4.73)	0.69 (0.20-2.44)
Model 2[b]	1.00	1.54 (0.67-3.54)	2.10 (0.93-4.73)	0.77 (0.22-2.71)
Men and women				
Person-years	41062	4743	9294	4674
Case, *n*	66	10	32	8
Age adjusted	1.00	1.14 (0.58-2.23)	1.82 (1.19-2.79)	0.94 (0.45-1.95)
Model 1[a]	1.00	1.12 (0.57-2.20)	1.63 (1.03-2.56)	0.79 (0.37-1.69)
Model 2[b]	1.00	1.12 (0.57-2.21)	1.62 (1.03-2.55)	0.69 (0.62-1.88)

TG, triglycerides; non-HDLC, non high-density lipoprotein cholesterol.
[a] Multivariable adjusted for age, body mass index, hypertension, diabetes, HDL (high-density lipoprotein) cholesterol, cigarette smoking and alcohol intake by a Cox proportional hazard model. Sex was also adjusted in the men and women combined model.
[b] Replacement of body mass index and hypertension (prevalence) as covariates in model 1 with waist circumference and systolic blood pressure levels.

of them in the future [28,29]. Second, we did not measure serum apolipoprotein B (apoB) [22], apolipoprotein A1 (ApoA1) and LP(a) [30], which some previous studies have shown to be strong risk factors for CAD [22]. Third, a recent study indicated that non-fasting TG is a better predictor of CAD than fasting TG [31]. However, in a large individual based meta-analysis in the Asia-Pacific region [26], most blood samples were collected during fasting, and there was a significant positive relationship between serum TG and CAD or stroke.

In conclusion, a combination of higher serum levels of TG and non-HDLC is associated with an increased risk of MI in a Japanese population. Furthermore, the risk for ischemic stroke was highest in the participants with high TG alone; however, further research should be needed. High serum levels of TG and non-HDLC are both important targets for the prevention of cardiovascular disease, which requires evidence-based guidelines for management in the primary care setting.

Acknowledgements

The present study was supported by grants-in-aid from the Ministry of Health, Labor and Welfare (H19-Seishu-017, H19-Seishu-021 and H20-Seishu-013). We sincerely appreciate members of the Suita Medical Foundation and Suita City Health Center. We thank researchers and co-medical staffs in the Department of Preventive Cardiology, National Cardiovascular Center, for their excellent medical examinations and follow-up surveys. We also thank *Satuki-Junyukai*, the society members of the Suita study.

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